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Interaction of hydrocortisone with ATP and adenosine on nerve-mediated contractions of frog skeletal muscle

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ABSTRACT

The inhibitory effects of ATP and adenosine on the nerve-mediated contractile responses of isolated sartorius muscle of the frog, *Rana ridibunda*, evoked by electrical field stimulation (EFS) were studied using pharmacological organ-bath technique. The effects of hydrocortisone applied *in vitro* and *in vivo* on contractility of sartorius muscle were also examined. ATP (100 μM) significantly reduced the amplitude of contraction to EFS of sartorius muscle, while pyridoxalphosphate-6-azonphenyl-2′,4′-disulfonic acid (PPADS; 10 μM), a P2 receptor antagonist, abolished inhibitory effect of ATP. A similar inhibitory effect of adenosine (100 μM) was fully antagonized by 8-(*p*-sulfophenyl)-theophylline (8-SPT, 100 μM), a P1 receptor antagonist. Incubation of the tissue with hydrocortisone (10 μM) caused a slight, but significant, decrease of muscle contractions. After incubation of muscle preparations with both hydrocortisone and ATP, no inhibition of muscle contractility was registered. A single injection of hydrocortisone (100 mg/kg) 12 h prior to experiments to frogs did not significantly change the nerve-mediated contractility of isolated sartorius muscle; however, it abolished the inhibitory action of ATP without changing inhibitory activity of adenosine. After treatment of frogs with hydrocortisone for 14 days (100 mg/kg/day), both ATP and adenosine retained their inhibitory action on EFS-induced contractions of the muscle, and their effects were antagonized by PPADS and 8-SPT, respectively. It is concluded that hydrocortisone has antagonistic actions against the inhibitory effects of ATP at the frog neuromuscular junction, although this effect is lost following long-term treatment with hydrocortisone.

1. Introduction

P2 receptors, which consist of a family of ligand-gated ion channel P2X receptors and a family of G-protein-coupled P2Y receptors, are widely distributed in animal and human tissues (Burnstock and Knight, 2004; Burnstock, 2007). These receptors are activated by ATP (the principal endogenous agonist) and some other natural purine and pyrimidine nucleotides and their analogs. It has been shown that P2 receptors are involved in regulation of many physiological processes in central and peripheral nervous systems, as well as functions of cardiovascular, urogenital, respiratory and gastrointestinal systems (Burnstock, 2006, 2007). In contrast to smooth muscle, in adult skeletal muscle, agonists and antagonists of P2 receptors directly cause neither contraction nor relaxation. However, it was found that ATP can modulate the effectiveness of neuromuscular transmission by prejunctional inhibition (Giniatullin and Sokolova, 1998; Galkin et al., 2001; Sokolova et al., 2003) or facilitation (Salgado et al., 2000) of transmitter release at the neuromuscular junction. ATP can also act as a postjunctional facilitator of transmission (Ribeiro, 1977).

It is widely accepted now that steroid hormones realize their effects not only by binding with specific intracellular receptors, but also by some other non-genomic ways (Haller et al., 2008; Song and Buttgereit, 2006). Non-genomic effects of steroids occur rapidly and can be due to interactions with membrane lipids, proteins, receptors and intracellular second-messenger systems (Watson and Gaetchu, 1999; Zor et al., 1991). At frog neuromuscular junction, the acute (non-genomic) effect of hydrocortisone was facilitation, while long-term treatment with this glucocorticosteroid was inhibition of multiquantal end-plate currents induced by motor nerve stimulation (Giniatullin et al., 2000).

The aim of this study was to evaluate the effects of hydrocortisone on P2 receptor-mediated inhibition of contractions of frog skeletal muscle.

2. Methods

2.1. General procedure

Experiments were carried out on *Rana ridibunda* frogs at room temperature (20–22 °C) during the months of September to March. The sartorius muscles were dissected free and suspended vertically for isometric recording of mechanical activity in 10 ml organ baths filled with the Ringer solution containing (in mM): NaCl 113.0; KCl

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